ERRATA

In Table I, last column heading, page 362 and in lines 17 and 19 from the top on page 363, "25.0 N KOH" should read "N/25 KOH".

Changes in Green Salted Calfskin Cured Under Aerobic and Anaerobic Conditions†

By L. S. STUART*

*Eastern Regional Research Laboratory***
Philadelphia, Pennsylvania

Many hides and skins received at the tannery have a butyric or propionic acid odor. However, in laboratory studies in which small pieces of calfskin were salted and then cured in humid chambers having loosely fitting covers, odors of this type did not develop. Investigation revealed that these odors are characteristic of old bedded hides or skins but not of hides and skins

* Now with the Livestock Branch, Production and Marketing Administration, United States Department of Agriculture.

** One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

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cured in a so-called "flat pack." In the latter, microbial activity is usually characterized by an ammoniacal odor similar to that developed in the usual small-scale curing study.

Preliminary experiments indicated that the predominant factor responsible for the butyric-propionic odors, instead of ammoniacal odors, was atmospheric or free oxygen. A study was made, therefore, to determine quantitatively the effect of atmospheric oxygen on the bacteriological and chemical changes taking place during the salt curing of animal skins.

In one experiment, 16 rectangular pieces of freshly flayed calfskin approximately 3.5 by 4 inches in size were salted on the flesh side with one-third their weight of specially prepared salt (two parts of new, grade G.A. salt and one part of used salt). Two pieces were then placed flesh side up, hair to flesh, on an inclined flat glass platform within each of eight shallow culture dishes. The culture dishes were put on an elevated glass shelf over water in individual humid chambers.[8] Four of these chambers were fitted with the usual loosely fitting covers. In the other four, ground raw potatoes were introduced beneath the elevated culture dishes, and the covers were sealed air-tight with rubber gaskets. The ground potatoes promoted rapid growth of aerobic bacteria, which removed the oxygen from the atmosphere. This is a slight modification of Torrey's method of producing anaerobic conditions in a sealed chamber, as described by Tanner.[9]

All eight humid chambers were placed in an incubator at 30° C. After 56 days they were opened, the drainings were collected, and 1 ml. aliquots were taken from them for bacteriological study. The salted skin pieces were then examined to determine their odor and physical condition. All the pieces aerobically incubated in the four unsealed chambers had a putrid strong ammoniacal odor; the flesh was red and slimy; and there was pronounced hair slip. All the pieces incubated in the four sealed chambers under anaerobic conditions had a pronounced butyric-propionic odor; the flesh was not discolored or slimy; and there was no hair slip.

After the pieces of skin were removed from the incubator, all eight lots were soaked for 24 hours in individual beakers, 6 mls. of water being used for each gram of skin. The soak waters were then combined with the collected drainings, and sufficient water was added to make 2 liters, after which aliquots were removed for chemical analyses.

Drainings from all of the calfskin samples were examined for aerobic and anaerobic bacteria. Bacterial counts for aerobic bacteria were made by the dilution plate method, both plain nutrient agar and agar with a high salt content being used. For anaerobic bacteria both the dilution plate method and the dilution tube method were employed, media with and without high concentrations of salt being used for both.

The media were made as follows:

Medium A—Plain Nutrient Agar (used for aerobic dilution plate counts)

Beef extract (Difco)	3 gms.
Peptone (Bacto)	10 gms.
Sodium chloride	10 gms.
Agar	15 gms.

Water to make one liter.
Adjust to pH 7.0 with 1.0 *N* NaOH.

Medium B—Gelatin High-Salt Agar (used for both aerobic and anaerobic dilution plate counts of halophilic and salt-tolerant bacteria)

Yeast extract	3.0 gms.
Peptone (Bacto-Tryptone)	10.0 gms.
Gelatin (Bacto)	60.0 gms.
Sodium thioglycollate	0.1 gm.
Sodium chloride	175.0 gms.
Agar	20.0 gms.

Distilled water to make one liter.
Adjust to pH 8.0 with 1.0 *N* NaOH.

Medium C—High-Salt Calcium Lactate Broth[4] (used for dilution tube counts of anaerobic halophilic and salt-tolerant bacteria)

Yeast water	500 mls.
Peptone (Bacto-Tryptone)	10 gms.
Gelatin (Bacto)	60 gms.
Glucose	1 gm.
Calcium lactate	5 gms.
Magnesium sulfate	1 gm.
Sodium chloride	175 gms.

Distilled water to make one liter.
Adjust to pH 8.0 with 1.0 *N* NaOH.

Medium D—Beef Heart Infusion Broth (used for dilution tube counts of anaerobic bacteria)

Extracted ground beef heart*	2 gms. per tube
Beef heart extract glucose broth**	10 mls. per tube

* Add 1 pound of ground beef heart to 1,000 mls. of distilled water; digest on a steam bath for 2 hours; press out extract; recover pressed residue.

** To the beef heart extract (see previous footnote), add 10 gms. of glucose, 10 gms. of NaCl, and enough distilled water to bring volume to 1 liter; adjust to pH 8.0 with 1.0 *N* NaOH.

TABLE I

RESULTS OF ANALYSES ON SOAK WATERS AND DRAININGS FROM SALT-CURED CALFSKIN

Incubated Under Aerobic or Anaerobic Conditions at 30° C.

Sample No.	Aerobic bacterial count		Anaerobic bacterial count			pH	Total nitrogen*	Protein nitrogen*	Ammonia nitrogen*	Ethyl Ether Soluble acids* Equiv. value 25*N* KOH
	Medium A dil. plate method	Medium B dil. plate method	Medium B dil. plate method	Medium C dil. tube method	Medium D dil. tube method					
	per ml†	per ml†	per ml†	per ml†	per ml†		mgms.	mgms.	mgms.	in mls.
				Cured under aerobic conditions						
1	5.5×10^6	1.3×10^5	3	1×10^3	1×10^7	7.5	18.6	3.2	8.4	0.10
2	2.3×10^6	6.2×10^4	1	1×10^3	1×10^8	7.6	19.8	2.7	8.2	0.10
3	1.8×10^7	7.1×10^5	0	1×10^3	1×10^{10}	7.6	12.0	4.5	6.3	0.08
4	4.4×10^7	2.2×10^4	3	1×10^3	1×10^7	7.8	19.7	3.0	8.4	0.08
Avg.	1.7×10^7	2.3×10^5	1.8	1×10^3	2.5×10^9	7.6	17.5	3.4	7.8	0.09
				Cured under anaerobic conditions						
5	2.8×10^3	2.0×10	4	1×10^8	1×10^{10}	5.8	11.8	9.2	2.1	0.15
6	5.1×10^2	0.6×10	2	1×10^8	1×10^4	4.7	10.5	6.3	2.3	0.20
7	2.7×10^2	2.3×10	0	1×10^8	1×10^7	4.5	14.9	9.2	2.1	0.20
8	7.6×10^3	1.4×10	0	1×10^9	1×10^5	5.5	13.1	8.3	2.6	0.15
Avg.	2.8×10^3	1.6×10	1.5	3.3×10^8	2.5×10^9	5.1	12.6	8.3	2.3	0.18

*Per gram of original green skin weight.

†Per ml. of undiluted drainings.

Media A and D were sterilized in an autoclave at 15 pounds pressure for 15 minutes, and Media B and C were sterilized at the same pressure for 10 minutes. The incubation temperature was 30° C. Aerobic culture incubations were conducted in humid chambers with loosely fitting covers. Anaerobic culture incubations were conducted in vacuum desiccators over pyrogallic acid in sodium carbonate solution. All cultures in media of high salt content were incubated for 30 days, whereas the cultures in low-salt media were incubated for only 96 hours.

A Beckman pH meter was employed in making all pH measurements reported in these studies. Total nitrogen values were obtained by the Kjeldahl-Gunning-Arnold method. Protein nitrogen values were obtained by precipitation with trichloroacetic acid and sodium chloride according to the procedure outlined by Highberger and Moore.[3] Ammonia nitrogen values were obtained by the method of Folin.[2] Fat in the combined soak waters and drainings was determined by washing aliquots in a separatory funnel with ethyl ether; the acid value of the fat was obtained by titration of the dried extract in alcohol with 25.0 *N* alcoholic KOH. Fat in the skin itself was determined by petroleum ether extraction, and the acid values were obtained by titration of the dried extract in alcohol with 25.0 *N* KOH.

Results of the analyses made on the soak waters and drainings of the eight lots of calfskins are given in Table I. This table shows that there were marked differences in the bacterial counts and chemical analyses, depending upon whether the cured skin pieces had been incubated under aerobic or anaerobic conditions. The aerobic dilution plate counts made in both low and high salt media were much higher for the aerobically cured skins than for the anaerobically cured skins. The anaerobic plate counts with Medium B and dilution tube counts with Medium D showed no significant differences between the anaerobic and aerobic samples. The dilution tube counts with Medium C, however, indicated that the number of anaerobic bacteria in the samples cured under anaerobic conditions was markedly greater than in the ones cured under aerobic conditions.

Chemical analyses of the soak waters and drainings showed that the reaction of those from the anaerobically cured skins was decidedly acid (average pH, 5.1), whereas for those of the aerobically cured skins it was slightly alkaline (average pH, 7.6). There was more total nitrogen in the aerobic cures than in the anaerobic cures. Only 20 per cent of it was protein nitrogen, however, whereas in the anaerobic cures 65 per cent of the total nitrogen was protein nitrogen. There was about 3 times as much ammonia nitrogen in the aerobic cures as in the anaerobic. Fifty per cent of the non-protein nitrogen in these cures (aerobic) was ammonia nitrogen, and in addition, appreciable but undetermined amounts of ammonia probably had been lost from the alkaline liquors by volatilization. The quantity of ethyl-

TABLE II

MOISTURE UPTAKE AND YIELDS OF CURED CALFSKIN IN CURING STUDIES

Conducted under Aerobic and Anaerobic Conditions at 30° C.

Sample No.	Initial weight of dry skin[1]	Weight of water absorbed during curing[2]	Weight of drained cured skin	Weight of drainings from cured skin	Wet weight of skin after soaking	Dry weight of soaked skin[3]	Yield of dry soaked skin[4]
	gms.	gms.	gms.	gms.	gms.	gms.	per cent
			Cured under aerobic conditions for 3 days				
1	55.7	2.0	169.7	42.3	191.2	61.2	109.8
2	55.7	5.0	167.4	47.6	194.0	62.4	112.0
3	54.5	4.7	158.3	51.0	192.4	60.3	110.6
Avg.	55.3	3.9	165.1	47.0	192.5	61.3	110.8
			Cured under aerobic conditions for 56 days				
4	55.7	74.6	163.9	120.7	171.8	48.2	86.5
5	55.7	77.6	159.8	126.8	172.0	46.9	84.2
6	54.5	73.1	164.3	118.9	177.3	46.5	85.3
7	54.5	63.8	161.1	121.8	169.2	52.8	96.8
Avg.	55.1	72.3	162.3	122.1	172.6	48.6	88.2
d[5]	−0.2	+68.4	−2.8	+75.1	−19.9	−12.7	−22.6
			Cured under anaerobic conditions for 56 days				
8	55.7	80.0	156.6	133.4	180.2	59.0	105.9
9	55.7	73.9	160.4	123.4	188.2	57.9	103.9
10	54.5	58.9	160.1	118.8	182.7	61.7	113.2
11	54.5	70.8	162.4	118.4	185.1	63.8	117.1
Avg.	55.1	70.9	159.9	123.5	184.1	60.6	110.0
d[5]	−0.2	+67.0	−5.2	+76.5	−8.4	−0.7	−0.8

[1]In mixtures of 150 gms. of diced, freshly flayed skin and 60 gms. of dry salt.
[2]Difference between initial green weight and final cured weight.
[3]Not salt-free.
[4]Based on weight of initial dry skin.
[5]d = deviation from average results obtained in 3-day cure for samples 1 .2 and 3

ether soluble free fatty acids appeared to be much smaller in the aerobic cures.

To confirm these results and obtain additional data, experiments were conducted by a number of methods. Complete quantitative data were obtained in two experiments in which composite samples of clipped, diced, freshly flayed calfskins were used. A special apparatus was used to collect drainings, allow for aeration, provide for maintenance of comparable moisture concentrations during incubation, and permit accurate periodic weighings without subjecting the samples to repeated handling. This apparatus, except for a large vacuum desiccator, is illustrated in Figure I. It consists of: a vessel (A), in which the mixture of skin and salt is placed for curing; a drain tube (B); an aerating manifold (D); an overflow tube (C); and a collecting vessel (E). Oxygen is introduced through the manifold to obtain aerobic conditions and is uniformly distributed by means of small orifices evenly spaced along the bottom of the manifold (Figure I). As salt-cured skin continually takes up water during storage at high humidities,[6] an overflow tube (C) is provided so that excess brine will drain into a vessel (E), from which it may be recovered for analysis. Weights of material used and of water absorbed during storage may be obtained by weighing the apparatus (1) when empty, (2) after filling and (3) during or at the end of the storage period. After the apparatus is filled, it is placed in a large vacuum desiccator, which provides control of humidity and supply of oxygen.

For tests in which aerobic conditions are used, continuous aeration is necessary. This is obtained by attaching the manifold, by means of a tube passing through an outlet in the desiccator, to a flask containing oxygen under slight positive pressure. Anaerobic conditions are obtained by removing oxygen from the air in the desiccator by a solution of sodium carbonate and pyrogallic acid, which is prepared and placed in the desiccator just before it is closed.

For these experiments, a clean but unwashed, freshly flayed calfskin was cut into ¼ inch cubes and thoroughly mixed. About 150 grams of the calfskin cubes and 60 grams of salt were then mixed and put into the vessel of the curing apparatus. This quantity filled the vessel nearly to the top of the overflow tube. After the necessary preliminary weights had been obtained, the apparatus was placed in a desiccator, which was then closed.

The pieces of skin were cured under anaerobic and under aerobic conditions for 56 days. For controls, or comparison pieces of skin in which bacterial action was at a minimum during curing, composite samples of skin were cured with the same amount of salt for 3 days under aerobic conditions. This time was considered to be long enough to allow for a fairly complete penetration of the salt and for the draining away of the salt-soluble skin proteins, yet too short to allow for detectable multiplication of bacteria.

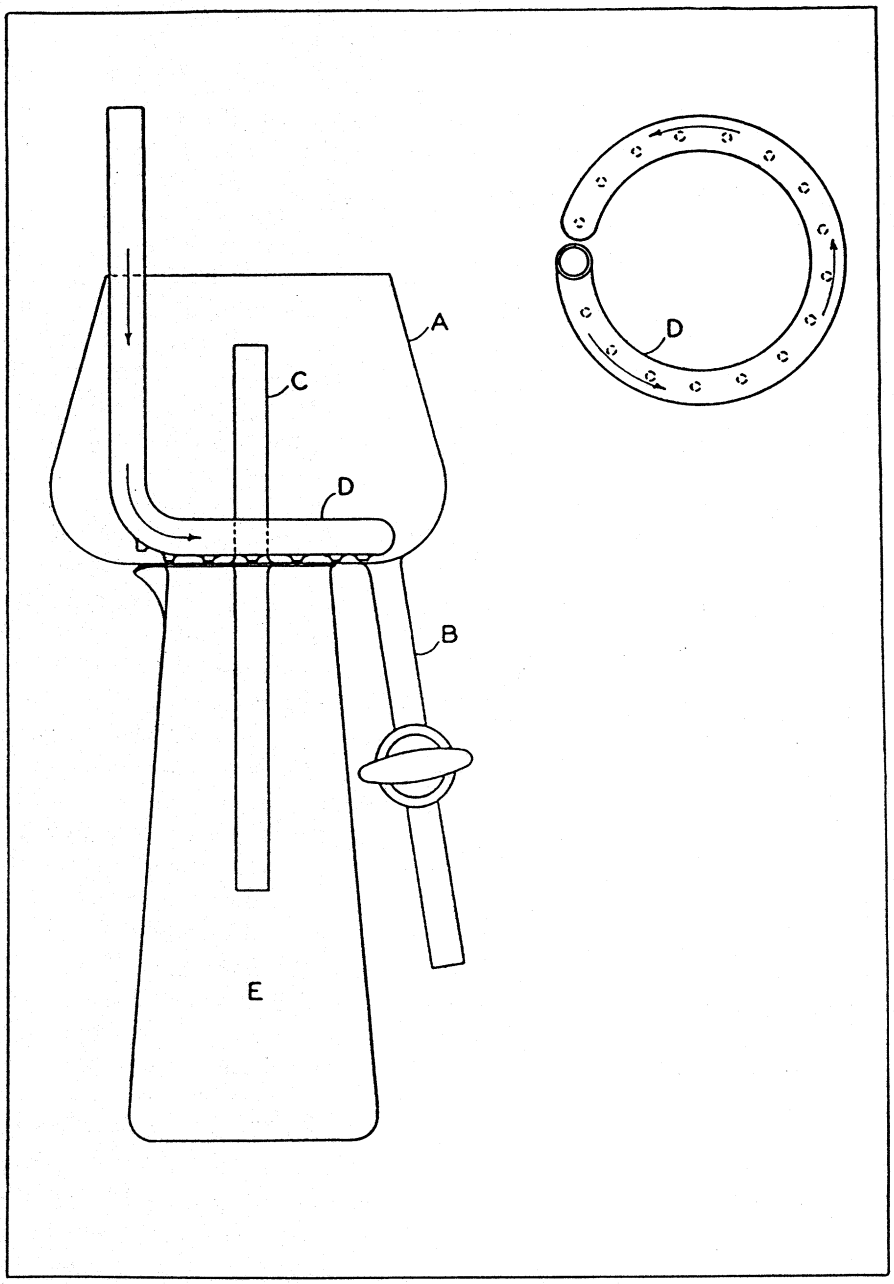

FIGURE I. Apparatus Used in Quantitative Studies of the Changes Occurring in Salted Calfskins Cured under Aerobic and Anaerobic Conditions. A, Vessel in which mixture of skin and salt is placed; B, Drain tube; C, Overflow tube; D, Aerating manifold; E, Collecting vessel.

In these studies, the salted pieces of skin soon became immersed in liquid brine. This was considered an advantage, since a moisture condition of this kind would tend to accelerate bacterial multiplication and accentuate chemical changes.

Weighings were made at the conclusion of the storage period without disturbing the assemblies as described (Figure I). The cured skin samples were then drained free of adsorbed moisture and liquid brine using the drain tube B (Figure I). These drainings were combined with the overflow collected during curing in vessel E (Figure I) and set aside for subsequent analyses. The drained cured skin samples in vessel A (Figure I) were then removed, weighed and placed in soak for 24 hours, 6 mls. of water being used per gram of skin.

The weights of the soaked skin samples were recorded after draining and after subsequent drying.

At the conclusion of the soaking period the soak waters and drainings were combined and brought to volume. Aliquots for both chemical and bacteriological analyses were then taken.

Medium C was the only medium employed in making the anaerobic dilution tube bacterial counts, for in the preliminary study, as shown in Table I, the most striking difference in anaerobic bacterial counts between the skins cured under aerobic and anaerobic conditions was in this medium. Medium B was selected for making the aerobic dilution plate counts because, although both Media A and B showed marked differences in bacterial count between aerobic and anaerobic cured skin, the results with Medium B were more uniform than those with Medium A. Direct microscopic counts were made as described in previous reports.[5, 7]

Total nitrogen, protein nitrogen, ammonia nitrogen, fat, fat acids, and pH values were determined by the same methods employed in the preliminary studies.

Table II shows the moisture uptake and yields of cured skin. The moisture uptake was approximately the same, regardless of whether the samples were cured under aerobic or anaerobic conditions. The average uptake for four samples cured under aerobic conditions for 56 days was 72.3 gms., as compared with 70.9 gms. for the corresponding samples cured under anaerobic conditions. The weights of the drained cured skins were also very nearly the same. However, the dry and wet weights of the samples after soaking indicate pronounced losses of hide substance in the samples cured under aerobic conditions.

Table III shows the comparative changes in protein content and in loss of hide substance of skins cured under aerobic and anaerobic conditions. That there were appreciable losses of hide substance from skins cured under aerobic conditions as compared with those cured under anaerobic conditions

TABLE III

PROTEIN CHANGES AND LOSS OF HIDE SUBSTANCE OF CALFSKIN

Cured under Aerobic and Anaerobic Conditions at 30° C.

Sample No.	Total nitrogen in drainings and soak waters	Protein nitrogen in drainings and soak waters	Nonprotein nitrogen in drainings and soak waters	Ammonia nitrogen in drainings and soak waters	Nitrogen in skin after soaking	Hide substance (T.N. x 5.62) in skin after soaking	Yield of hide substance[1]
	gms.	gms.	gms.	gms.	gms.	gms.	per cent
			Cured under aerobic conditions for 3 days				
1	0.525	0.389	0.136	0.071	7.019	39.447	70.82
2	0.506	0.419	0.087	0.081	7.225	40.605	72.89
3	0.594	0.419	0.175	0.042	6.722	37.778	69.32
Avg.	0.542	0.409	0.133	0.065	6.989	39.277	71.01
			Cured under aerobic conditions for 56 days				
4	0.942	0.174	0.768	0.308	5.881	33.051	59.34
5	0.963	0.158	0.805	0.346	5.966	33.329	59.84
6	1.101	0.147	0.954	0.724	5.870	32.692	59.98
7	1.561	0.223	1.338	0.697	6.209	34.895	64.03
Avg.	1.142	0.176	0.966	0.519	5.982	33.739	60.80
d[2]	+0.600	−0.233	+0.833	+0.454	−1.007	−5.538	−10.21
			Cured under anaerobic conditions for 56 days				
8	0.643	0.365	0.278	0.110	6.734	37.845	67.94
9	0.647	0.344	0.303	0.211	6.343	35.648	64.00
10	0.652	0.403	0.249	0.093	6.611	37.154	68.91
11	0.674	0.459	0.215	0.069	6.638	37.306	68.43
Avg.	0.654	0.393	0.261	0.121	6.582	36.988	67.32
d[2]	+0.112	−0.013	+0.128	+0.056	−0.407	−2.289	−3.69

[1]Based on initial dry weight of freshly flayed skin. See Column 2 of Table II.
[2]d = deviation from average results obtained in 3-day cure for samples 1, 2 and 3

is borne out by the results for total nitrogen, nonprotein nitrogen, and ammonia nitrogen.

This table shows clearly that nearly twice as much total nitrogen was lost in the soak waters and drainings from skins cured under aerobic conditions as from those cured under anaerobic conditions. The average amounts of protein nitrogen, nonprotein nitrogen and ammonia nitrogen in the soak waters and drainings of the skins cured for 56 days under anaerobic conditions were not much different from those from samples cured for 3 days under aerobic conditions (control). On the other hand, the protein nitrogen values were much lower, and the nonprotein nitrogen and ammonia nitrogen values were much higher, for the samples cured for 56 days under aerobic conditions. As in the preliminary study, there was probably some loss of ammonia nitrogen in the aerobic cures, owing to volatilization from the alkaline liquors. The total nitrogen recovered in the soaked skin was correspondingly lower for the skin samples cured under aerobic conditions. The average yield of hide substance for these samples was 10.21 per cent less than the average yield for the 3 day controls. The yield of hide substance for the skins cured under anaerobic conditions was only 3.69 per cent less than that of the controls. These results indicate clearly that curing under the aerobic conditions employed in these studies was accompanied by a marked protein digestion, including deamination. Under anaerobic conditions, proteolysis was not pronounced.

In Table IV are given the pH values, bacterial counts, and fat contents of calfskins cured under aerobic and anaerobic conditions. Confirming the data shown in Table I, the pH values for the skins cured under aerobic conditions were much higher than those of the 3 day control samples; the pH values of the skins cured under anaerobic conditions were much lower than those of the controls. The samples cured under aerobic conditions showed marked increases in the aerobic dilution plate and direct microscopic bacterial counts, indicating fairly conclusively that the proteolysis observed with these samples was due to bacterial activity. On the other hand, the direct microscopic bacterial counts on the samples cured under anaerobic conditions also showed marked increases, and this was accompanied by increases of a similar magnitude in the anaerobic dilution tube count in Medium C. It would appear, therefore, that the bacteria which developed under anaerobic conditions were nonproteolytic types.

Attempts to isolate and identify the bacteria which developed anaerobically in Medium C were not successful. Transfers from the initial cultures in very high dilutions failed to grow, and transfers in low dilutions died out after about 6 serial transfers. It would appear from this that Medium C lacked some essential growth factor for these organisms.

TABLE IV

CHANGES IN pH, BACTERIAL COUNT, AND FAT IN CALFSKIN

Cured under Aerobic and Anaerobic Conditions at 30° C.

Sample No.	pH		Bacterial Counts[1]			Total fat in skin after soaking[2]	Total fat in combined drainings and soak waters[2]	Acid number of fat in skin after soaking	Acid number of fat in combined drainings and soak waters
	drainings	soak waters	Aerobic dil. plate method Medium B	Anaerobic dil. tube method Medium C	Direct microscopic count				
			per gm.	per gm.	per gm.	gms.	gms.	mg. KOH/ gm. fat	mg. KOH/ gm. fat
				Cured under aerobic conditions for 3 days					
1	6.30	6.40	1.1×10^4	6.7×10^5	2.8×10^6	14.925	2.549	19.3	23.9
2	6.35	6.50	1.3×10^4	6.7×10^5	2.8×10^6	14.175	2.205	27.4	27.2
3	6.45	6.60	3.4×10^3	5.0×10^4	2.6×10^6	13.185	0.900	25.4	26.6
Avg.	6.37	6.50	9.1×10^3	4.6×10^5	2.7×10^6	14.095	1.885	24.0	25.9
				Cured under aerobic conditions for 56 days					
4	7.72	7.50	2.1×10^6	6.7×10^4	4.3×10^7	11.895	3.279	70.5	21.2
5	7.84	7.70	5.9×10^5	6.7×10^5	5.1×10^7	9.585	3.429	71.0	20.3
6	7.25	7.35	2.8×10^5	5.0×10^4	3.4×10^9	8.505	3.945	107.7	26.5
7	7.35	7.40	1.9×10^5	5.0×10^4	4.2×10^8	8.460	3.660	109.4	27.1
Avg.	7.54	7.49	7.9×10^5	2.1×10^5	9.8×10^8	9.611	3.578	89.7	23.8
d[3]	+1.17	+0.99	$+7.8 \times 10^5$	-2.5×10^5	$+9.8 \times 10^8$	−4.484	+1.693	+65.7	−2.1
				Cured under anaerobic conditions for 56 days					
8	4.95	5.30	3.9×10^2	6.7×10^7	2.1×10^8	15.420	1.806	16.4	105.0
9	5.17	5.45	1.8×10^2	6.7×10^9	1.8×10^8	12.645	2.790	15.9	94.8
10	5.05	5.65	8.0×10^1	5.0×10^6	1.7×10^6	13.140	2.022	37.0	76.5
11	5.12	5.35	2.7×10^3	5.0×10^8	2.9×10^7	11.805	3.090	41.3	87.0
Avg.	5.07	5.44	8.4×10^2	1.7×10^9	1.1×10^8	13.253	2.427	27.7	90.8
d[3]	−1.30	−1.06	-8.3×10^3	$+1.7 \times 10^9$	$+1.1 \times 10^8$	−0.842	+0.542	+3.7	+64.9

[1]Per gram of initial green skin weight.
[2]See Table II for initial skin weights.
[3]d = deviation from average results obtained in 3-day cure with samples 1, 2 and 3.

Inasmuch as strong propionic and butyric odors developed in all skin samples cured under anaerobic conditions, it might be contended that these anaerobic nonproteolytic bacteria developed at the expense of the animal skin fat. From the data given in Table IV, this does not seem to be the case. Fat recovered from the skin samples cured for 56 days under anaerobic conditions was on the average only slightly less than for the 3 day controls. Much less fat was recovered from the samples cured for the same length of time under aerobic conditions. The amounts of fat recovered from the soak waters and combined drainings of the skins cured under anaerobic conditions were not appreciably different from the amounts recovered from the 3 day control samples. In the skins cured under aerobic conditions, a larger part of the fat which disappeared from the samples during soaking was recovered from the soak waters. This might indicate that proteolysis in these samples resulted in a slightly greater physical release of the skin fat. The acid numbers of the fats extracted from the soaked skin samples and from the combined drainings and soak waters are of special interest. The residual skin fat of the samples cured for 56 days under aerobic conditions had excessively high acid numbers, but the fat fractions recovered from the soak waters had low acid numbers. The picture with the samples cured for 56 days under anaerobic conditions was completely reversed. The residual skin fat had low acid numbers, but the fat fractions recovered from the soak waters had very high acid numbers. Such results cannot be easily explained. The excessively high values for the residual skin fat in the one case and for the soak-water fat extract in the other indicate the presence of large amounts of short-chain free fatty acids. In the samples cured under aerobic conditions, it would appear that these were formed at the expense of the skin fat and that those which were extracted into the soak water may have been neutralized by the free ammonia present, which formed soluble salts that were not recovered in the ether extract. In the samples cured under anaerobic conditions, it would appear that these acids were formed as the result of a fermentative breakdown of some skin constituent other than fat. Since little or no free ammonia was formed, and the acids remained unneutralized in the soak waters, a fairly quantitative recovery in the ether extract should have resulted.

A special study was made to determine empirically the comparative effect of aerobic and anaerobic curing on skin collagen. Samples were prepared from skin cured with salt at 30° C. for 3 and 56 days under aerobic conditions, and for 56 days under anaerobic conditions. The following procedure was used.

Pieces of the cured skin were soaked for 24 hours at 20° C., the water being changed three times, at 8 hour intervals. The samples were then transferred to a saturated solution of lime water for 72 hours at 25° C., after which the

reticular and grain layers were removed by slicing with a razor. All pieces of corium were delimed in dilute acetic acid and washed in three 2 hour changes and one 18 hour change of distilled water. Each sample was drained and dried in a vacuum desiccator, ground in a Wiley mill, resuspended in distilled water, treated a second time with dilute acetic acid, washed free of acetic acid with distilled water, dehydrated with 95 per cent alcohol, washed in ethyl ether, and dried in a vacuum desiccator. From each sample prepared as described above, a 500 mgm. portion was taken for Van Slyke[11] nitrogen distribution determinations, for which the microprocedure outlined by Cavett[1] was used.

Table V shows the basic amino nitrogen in the limed collagen from skins cured under aerobic and anaerobic conditions. The collagen sample from skin salt-cured for 56 days under aerobic conditions contained 2.4 per cent less basic amino acid nitrogen than that prepared from the control skin cured for 3 days, and 1.5 per cent less than that from skin salt-cured for 56 days under anaerobic conditions. These differences in the values found for the basic amino acid nitrogen were of the same magnitude as the differences found in the total nitrogen values. It would appear therefore that all the nitrogen lost from the collagen during curing was basic amino acid nitrogen.

TABLE V

BASIC AMINO ACID NITROGEN IN LIMED COLLAGEN FROM CALFSKIN
Cured under Aerobic and Anaerobic Conditions

Source of sample	Size of sample	Total nitrogen	Basic amino acid nitrogen	Per cent total nitrogen accounted for as basic amino acid nitrogen
	mgms.	mgms.	mgms.	
Skin salt-cured under aerobic conditions for 3 days (control).....	500	79.20	30.15	38.0
Skin salt-cured under aerobic conditions for 56 days.....	500	76.55	27.23	35.6
Skin salt-cured under anaerobic conditions for 56 days.....	500	78.10	28.88	37.1

Whether the differences observed in the composition of the limed samples of collagen shown in Table V are great enough to bring about significant differences in the subsequent combining power of the skin for formaldehyde, or basic chrome and vegetable tan liquors, is not known. Liming brings about a deamination of collagen,[10] and a limewater extraction was used in the preparation of the samples for this study. All samples were limed in exactly the same manner, however, and it would appear, therefore, that the collagen of the skin cured under aerobic conditions for 56 days had suffered consid-

erable deamination prior to the limewater treatment. The results suggest that skins cured under aerobic conditions might process considerably different from those cured under anaerobic conditions.

A number of preliminary experiments were conducted to determine the influence of curing, under aerobic and anaerobic conditions, on the formation of permanent grain stains. In these studies small pieces of freshly flayed calfskin were salted and then put into large humid chambers over water and incubated for varying periods of time under (1) aerobic or (2) anaerobic conditions, (3) anaerobic conditions following aerobic conditions, and (4) aerobic conditions following anaerobic conditions. Pieces of freshly flayed

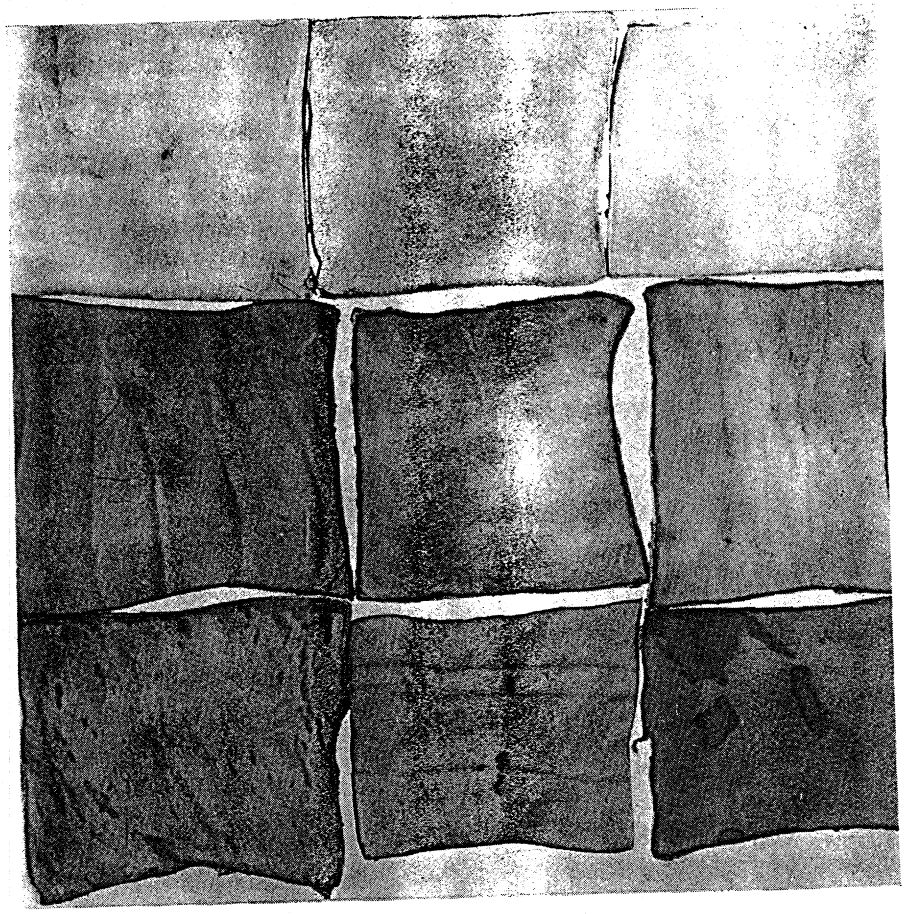

FIGURE II. Stains on Calfskins that were Dipped in Blood and then Salt-Cured under Aerobic and Anaerobic Conditions at 30° C.
Top Row—Cured aerobically for 56 days.
Middle Row—Cured anaerobically for 56 days.
Bottom Row—Cured anaerobically for 28 days, then aerobically for 28 days.

calfskin dipped in fresh whole calf blood prior to salting and pieces dipped in blood and stored for varying lengths of time prior to salting were also studied in this manner.

The pieces of freshly flayed calfskin that were salted and cured under either anaerobic or aerobic conditions for 56 days did not show grain stains when soaked, limed, depilated, and pickled. When the skins were cured anaerobically for 28 days and then aerobically for another 28 days, a few blue stains were found on the grain of the depilated skin. These stains were not removed in a sulfuric acid-salt pickle. When the skins were cured aerobically for 28 days and then for 28 days under anaerobic conditions, similar stains were not found.

Stains did not develop on freshly flayed skins that had been dipped in whole calf blood prior to salting and cured aerobically for 56 days. Pieces cured anaerobically for 56 days did not show stains but were uniformly dull gray in color even after they were pickled in a sulfuric acid-salt pickle. When such pieces were cured anaerobically for 28 days and then aerobically for 28 days, permanent dark-blue stains developed on the grain. These were not removed during pickling. The stains described are shown in Figure II. No stains were found on the pieces dipped in blood prior to salting and cured for 28 days under aerobic conditions and then for 28 days under anaerobic conditions.

These few results do not adequately show the possible effects of aerobic and anaerobic conditions of curing on stain formation. They indicate strongly, however, that oxidative changes following anaerobic curing conditions may result in permanent staining, especially when blood is present. They suggest further that a study of chemical changes brought about in hemoglobin by bacteria under aerobic and anaerobic conditions, particularly with regard to the activity of the organically bound iron compounds produced, might throw considerable light upon the formation of grain stains.

Summary and Conclusions

Studies were conducted to determine the effect of oxygen on the development of butyric-propionic acid odors, on bacterial growth, on chemical changes, and on formation of grain stains during the salt curing of hides and skins.

It would seem from the results obtained that butyric-propionic odors in cured hides and skins indicate a cure under anaerobic conditions, whereas a strong ammoniacal odor is strong presumptive evidence of an aerobic type of cure.

Calfskins developed a slightly alkaline reaction under aerobic conditions in salt curing, as compared with a pronounced acid reaction under anaerobic conditions.

Aerobic dilution-plate bacteria counts, in both low and high salt media, and direct microscopic counts increased when calfskins were salt-cured under aerobic conditions. Under anaerobic conditions, there was an increase in anaerobic dilution-tube bacterial counts, in which Medium C (high-salt calcium lactate broth) was used, and in the direct microscopic counts. It would appear that the anaerobic bacteria in salted hides, which grow in Medium C, were responsible for the acid produced in the anaerobic cure.

Calfskins salt-cured under aerobic conditions lost considerably more hide substance than those salt-cured under anaerobic conditions, indicating that bacterial proteolysis is greater under aerobic conditions. A further indication of this is the fact that soak waters and drainings from the aerobically cured skins contained more ammonia and nonprotein nitrogen and less protein nitrogen than did soak waters and drainings from salted calfskins cured under anaerobic conditions.

More ether-soluble acids were found in the soak waters and drainings of anaerobically cured skin than in the soak waters and drainings of aerobically cured skin. It is probable that these were produced by the action of anaerobic bacteria on the carbohydrate fractions of the skin, since analyses showed there was little or no loss of fat or increase in free fatty acids in the anaerobically cured and soaked skins.

Skins salt-cured under aerobic conditions showed a greater loss of fat and a larger increase in free fatty acids than skins salt-cured under anaerobic conditions.

Chemical analyses of especially prepared samples of collagen from green salted calfskins cured under (a) aerobic conditions for 3 days (control), (b) aerobic conditions for 56 days and (c) anaerobic conditions for 56 days showed that the greatest amount of deamination occurred in the skins cured under aerobic conditions for 56 days. This deamination appeared to have taken place entirely at the expense of the basic amino acids. These results suggest that the chemical differences between skins cured under aerobic and anaerobic conditions may be great enough to result in subsequent variations in the affinity of the hides and skins during processing for different tanning materials. Further studies will be necessary to evaluate just how important such differences may be to the commercial tanner.

Evidence obtained in these studies suggests that salted calfskins cured anaerobically may be susceptible to staining by the subsequent growth of aerobic microorganisms, especially when blood is present.

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Discussion

R. M. LOLLAR: This paper is particularly interesting when we consider the implications that this work brings forth with respect to practical tannage—the possibility that the variations in the soaking conditions under different methods of handling the skins would produce different types of result in cured skins which would then tan differently. I wonder if some of the practical tanners here might not have observed that in their own experience.

There is one question I would like to ask about the paper and that is whether any histological data are available as to whether any differences can be noted by this technique under the two methods of cure?

I. D. CLARKE: As far as I know, no. There are none in the paper and I think probably none were made.

LOLLAR: Does anyone on the floor have discussion on this paper? There are a number of practical implications in the paper and perhaps previous observations have been made along these lines—when you consider there would be different aerobic and anaerobic conditions in a pack of hides, depending on how the pack was formed.

H. G. TURLEY: I think it would be instructive to examine the residual liquor for the presence of amines and ammonia. It is suggested because it was that idea that led McLaughlin and his associates to that arrangement—the hypothesis that amines were produced by bacterial action on the raw skin. I think it would be very instructive to lime such cured skins under different conditions and observe the speed with which the unhairing process then proceeds.